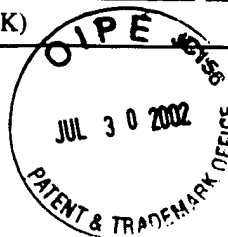


Appendix C

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Δ 7-Sterol-C5-desaturase: molecular characterization and functional expression of wild-type and mutant alleles

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Abstract

An *Arabidopsis thaliana* recessive monogenic mutant (*ste1-1*) presenting a deficiency of the Δ 7-sterol-C5(6)-desaturase step in the sterol pathway has been reported previously [12]. To further characterize *ste1-1*, *Arabidopsis*, *Nicotiana tabacum* and *Homo sapiens* cDNAs encoding Δ 7-sterol-C5(6)-desaturases were isolated and identified on the basis of their ability to restore ergosterol synthesis in *erg3*, a yeast null mutant whose gene encoding the Δ 7-sterol-C5(6)-desaturase was disrupted. Overexpression of the *Arabidopsis* cDNA driven by a 35S promoter in transgenic *ste1-1* plants led to full complementation of the mutant. This result demonstrates that STE1 was the impaired component in the desaturation system. Four independent reverse transcriptions of *ste1-1* RNA followed by polymerase chain reactions (RT-PCRs), yielded a single product. Alignment of the wild-type ORF with the RT-PCR derived *ste1-1* ORF revealed a single amino acid substitution: Thr-114 in the wild-type is changed to Ile in *ste1-1*. Expression in *erg3* resulted in a 6-fold lowered efficiency of the *ste1-1* ORF in complementing the yeast biosynthetic pathway when compared to the wild-type ORF. The presence of this mutation in the mutant *ste1-1* genomic sequence (and no additional modification between *ste1-1* and wild-type genes) demonstrates that the change of the Thr-114 to Ile is necessary and sufficient to create the leaky allele *ste1-1*. The occurrence of a hydroxylated amino acid (Thr or Ser) at the position corresponding to Thr-114 in the five Δ 7-sterol-C5(6)-desaturases identified so far suggests that this amino acid is important for normal enzymatic function.

Keywords

sterol, Δ 7-sterol-C5(6)-desaturase, deficient mutant, complementation, *Arabidopsis*

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